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(54) Title: MUTANTS OF O⁶-ALKYLGUANINE-DNA ALKYLTRANSFERASE

WO 2005/085431

(57) **Abstract:** The invention relates to AGT mutants showing, when compared to the wild type human AGT, two or more advantageous properties selected from (a) reduced DNA interaction; (b) localisation of the expressed protein in eukaryotic cells that is no longer restricted to the nucleus; (c) improved expression yield as soluble protein and improved stability in various hosts; (d) improved stability under oxidising conditions; (e) improved stability within cells after reaction with a substrate; (f) improved stability outside cells before and after reaction with a substrate; (g) improved *in vitro* solubility; (h) improved reactivity against O⁶-alkylguanine substrates; (i) reduced reactivity against DNA-based substrates; and (j) reduced reactivity against N⁹-substituted O⁶-alkylguanine substrates. Such AGT mutants with the mentioned improved properties are mutants wherein between 1 and 25 amino acids of the wild type human AGT are substituted by other amino acids, and optionally 1 to 5 amino acids out of the continuous chain at one, two or three positions are deleted or added and/or 1 to 4 amino acids at the N-terminus or 1 to 40 amino acids at the C-terminus are deleted. The invention further relates to a method for detecting and/or manipulating a protein of interest wherein the protein of interest is incorporated into a fusion protein with the AGT mutants of the invention. Another object of the invention are AGT fusion proteins comprising such AGT mutants and the protein of interest.